REMARKS

The Office Action

Claims 1-39 are pending. Claims 1-6 and 25-38 stand rejected for lack of enablement. Claims 1-6, 25-32, and 35-38 stand further rejected for anticipation by Good et al. (U.S. Patent Publication No. 2002/0069423; hereafter "Good"). Applicants request clarification of the status of claim 39.

Support for Amendments

By the present amendment, claims 1, 5, 25, 29, 32, and 37 have been amended. These amendments find support in the specification, for example, as follows: Claim 1, original claim 2; claim 5, original claim 3; claim 25, original claim 26; claim 29, original claim 27; claim 32, original claim 34 and Example 1, page 54; claim 37, page 16, lines 19-27. These amendments add no new matter.

For the record, Applicants reserve the right to pursue all cancelled subject matter in this or a future, related application.

Examiner Interview

Applicants thank the Examiner for an extremely helpful interview on April 5, 2007 and submit this reply in furtherance of that interview.

Specifically, as recommended by the Examiner, claims 32 and 37 (and their dependent claims) have been amended to include steps that further distinguish the claimed method from those described in the cited reference by Good. Claim 32 now requires, as part of the multiple prion targeting approach, that bovines cell harboring a first targeting vector be isolated as individual colonies prior to introduction of a second targeting vector. This step, which is not enabled by Good, is important to the success of Applicants' prion knockout approach. In addition, claim 37 has been amended to require that cloning of the prion knockout bovine be accomplished by fusion of a permeabilized

donor cell to a metaphase II oocyte. This approach is neither taught nor suggested by Good, again distinguishing claim 37 from the disclosure of the Good reference.

Rejections under 35 U.S.C. § 112, first paragraph

Claims 1-6 and 25-38 stand rejected for lack of enablement. As stated in M.P.E.P. § 2164, "[t]he purpose of the requirement that the specification describe how to make and use the claimed invention is to ensure that the invention is communicated to the interested public in a meaningful way." Applicants have met this standard.

In reply to the September 2006 Declaration of Dr. Kuroiwa submitted in response to the previous enablement rejection, the Examiner raises two further objections as to its sufficiency: (1) the "declaration is not clear as to the vectors used in the production of the calves taught in the declaration" and (2) "it is not possible to know if the particular cell lines used to produce the bovines of the declaration were critical."

As an initial matter, Applicants point out that claims 25-36 are directed to bovine cells and methods of producing bovine cells. Experimental data demonstrating the production of heterozygous and homozygous PrP knockout bovine cells is provided in the specification. Therefore, it is believed that neither of the objections to the September 2006 Declaration raised by the Examiner is relevant to production of bovine cells. As the application provides methods that result in the actual production of PrP knockout bovine cells, and the Office has not raised any objection to the sufficiency of those data, the rejection of claims 25-36 may be withdrawn.

With respect to the two objections regarding Dr. Kuroiwa's Declaration, Applicants first note that the September 2006 Declaration provided information regarding the vectors employed. For example, the September 2006 Declaration, at ¶ 4, refers to a published paper (Kuroiwa et al, *Nat. Genet.* 36: 775-780 (2004)) for the methods used to produce prion knockout cows. The vectors referred to in ¶ 4 are described in Kuroiwa et al. at page 778-779 and Figure 4, and a copy of this paper was submitted as part of the Declaration. This Declaration further identified the vectors employed by the same

designation as that used in the specification. Thus, in response to the Office's concern, Applicants confirm that the September 2006 Declaration did identify the vectors used to produce living PrP knockout cows, vectors that are the same as those disclosed in the application.

Nonetheless, as further support, Applicants enclose an additional Declaration of Dr. Kuroiwa that provides further detail on the methods employed to produce living PrP knockout cows and provides citations where corresponding methods may be found in the specification (June 2007 Declaration ¶ 4). As stated by Dr. Kuroiwa, to produce prion knockout bovines, a male Holstein primary fetal fibroblast line, 6594, was transfected with the first and second KO vectors (pBPrP(H)KOneo and pBPrP(H)KOpuro vectors) to sequentially disrupt the two alleles of the PrP gene as described in Kuroiwa et al. (Sequential targeting of the genes encoding immunoglobin-μ and prion protein in cattle. *Nat Genet.* 36: 775-780 (2004)). The structure of these vectors is shown in Exhibit 1 of the June 2007 Kuroiwa Declaration. This Exhibit is substantially the same as Figures 44A-44B of the specification. Dr. Kuroiwa further states that the pBPrP(H)KOneo and pBPrP(H)KOpuro vectors employed in the experiments described in the June 2007 Declaration are the same as the STOP codon-containing vectors described in the specification (page 55, line 13 to page 56, line 2). Primary fetal fibroblast line 6594 was electroporated at 550 V and 50 μF with the first KO vector (pBPrP(H)KOneo) (as substantially taught at specification page 56, lines 5-17). Ninety-four colonies resistant to G418 (500 μg/ml) were screened by PCR to identify homologous recombinants (primer pair; neoF7 x neoR7) (40/94: 43 %) (as substantially taught at specification page 56, line 19 to page 57, line 25). Based on their morphology, seven colonies were selected and subjected to embryonic cloning to generate fetuses (as substantially taught at specification page 58, lines 1-26). At 40-60 days of gestation, five fetuses were collected and three of them (2180, 3560-1, and 3560-2) were confirmed to be $PrP^{+/-}$ (primer pair; neoF7 x neoR7). The heterozygous $PrP^{+/-}$ cell line, 3560-2, was electroporated with the second KO vector (pBPrP(H)KOpuro), and 182 colonies resistant to puromycin (1 μg/ml) were

screened by PCR (primer pair; puroF14 x puroR14) to identify homozygously targeted colonies (as substantially taught at specification page 58, line 28 to page 60, line 12). Six colonies were identified to be $PrP^{-/-}$, four of which were used for embryonic cloning to generate recloned fetuses (as substantially taught at specification page 60, lines 12-21, Table 1). At 40-75 days of gestation, 10 fetuses were collected, and then fibroblast cell lines were established (as substantially taught at specification page 60, lines 12-18). All of the cell lines were confirmed to be homozygous $PrP^{-/-}$ by targeting event-specific PCR analysis (puroF14 x puroR14 and neoF7 x neoR7) and prion-negative by PCR analysis (BPrPex3-F x BPrPex3-R) (as substantially taught at specification page 61, lines 1-17). Three of the $PrP^{-/-}$ fetal cell lines (5211, 5232, and 4296) were recloned to produce calves. As stated by Dr. Kuroiwa, five (16 %), two (8.6 %), and five (26 %) calves were obtained from the cell lines 5211, 5232, and 4296. These experiments demonstrate that the methods described in the specification may be, and were, employed to successfully produce living PrP knockout bovines. This first basis for the rejection may be withdrawn.

With respect to the objection regarding the cell lines employed, Dr. Kuroiwa states at ¶ 4 of the June 2007 Declaration that the specification reports that four PrP homozygous knockout cell lines (1395, 4661, 1439, and 1487-1) resulted in viable pregnancies at 90 days (page 60, Table 1). As further noted, these four cells lines were derived from heterozygous PrP cell line 3560-1, which was derived from male Holstein primary fetal fibroblast line, 6594. None of the particular pregnancies reported in the specification resulted in live birth of a calf, but, as Dr. Kuroiwa states, certain cell lines, including primary, non-cloned cell lines, do not always result in live births. Dr. Kuroiwa further opines that the lack of live births using cells derived from cell line 3560-1 was the result of an animal cloning effect and was not a gene targeting error. This view is supported by the successful production of live PrP homozygous knockout calves using cells derived from cell line 3560-2, which is genetically identical to 3560-1, and the same methods.

In summary, the homozygous PrP knockout cell lines reported in the September 2006 Declaration and the specification were derived from two genetically identical heterozygous PrP knockout cell lines (3560-1 and 3560-2), which were in turn derived from cell line 6594. Living PrP knockout cows have been produced using homozygous cell lines derived from cell line 3560-2.

With respect to the criticality of the cell line employed, the June 2007 Declaration of Dr. Kuroiwa states at ¶ 8 that the cell line is not critical. This view of Dr. Kuroiwa is supported by the specification at page 57, lines 20-25, where Applicants report that two different fibroblast cell lines could be used to produce heterozygous PrP knockout cells.

As the cell line employed in the cloning process is not critical to the success of producing living PrP knockout cows, this second basis of the rejection may also be withdrawn.

Rejection under 35 U.S.C. § 102

Claims 1-6, 25-32, and 35-38 stand further rejected for anticipation by Good. As stated in the M.P.E.P., "The disclosure in an assertedly anticipating reference must provide an enabling disclosure of the desired subject matter; mere naming or description of the subject matter is insufficient, if it cannot be produced without undue experimentation" (M.P.E.P. § 2121.01; citations omitted). The anticipation rejection in this case should be withdrawn because Good is not an enabling disclosure.

Applicants have previously submitted a Declaration by Dr. Kuroiwa on the deficiencies of the Good reference. In response, the Office has raised three objections: (1) Applicant "has not distinguished the present claims from the disclosure of Good," (2) Applicant has not "explained what methodology they used provided PrP calves," and (3) "the proper course of action is to request an interference."

In support of the first objection, the Office has cited the case of *In re Crosby*, 157 F.2d 198 (CPPA 1946) (M.P.E.P. § 716.07) (copy enclosed). This case involved the rejection of claims directed to staplers for anticipation by the disclosure of an inoperative

stapler in a prior patent. While assuming that the prior art stapler was inoperative, the court maintained the rejection because the inoperative stapler was nonetheless encompassed by the rejected claims. Implicit in the court's reasoning was that the inoperative stapler could be made – it just would not work as a stapler.

The present case is distinguishable from the facts of *In re Crosby* because Applicants' claims do not cover inoperative embodiments disclosed by Good. Claim 1 requires a PrP knockout bovine; claim 26 requires a PrP knockout bovine cell; claim 32 requires the production of a PrP knockout bovine cell; and claim 37 requires the production of a PrP knockout bovine fetus. Good does not describe how to make inoperative forms of these embodiments (analogous to the inoperative stapler in *Crosby*). Instead, Good fails entirely to report the production of either heterozygous or homozygous PrP knockout bovine cells or bovines. As noted by Dr. Kuroiwa in his Declaration regarding Good, Good failed in its actual attempts to produce PrP knockout cells (Dr. Kuroiwa's Good Declaration ¶ 3) and provides insufficient prophetic guidance to do so (Dr. Kuroiwa's Good Declaration ¶ 4).

As further support, Applicants' enclose a Declaration of Dr. Robl regarding additional deficiencies in the teachings of Good. As stated by Dr. Robl, if one skilled in the art followed the methods outlined in the Detailed Description (including the Examples) of Good, it would not reproducibly result in the production of a PrP) knockout cell or bovine (Dr. Robl's Declaration ¶ 2). In support of his opinion, Dr. Robl states that Good provides a single, prophetic method for producing PrP knockout cows (Example 2). In this proposed method, Good describes a first targeting step in paragraph 165, in which bovine fetal fibroblasts (BFF) are electroporated in the presence of a targeting vector and then plated in 100 mm^2 culture dishes at a density of 500,000 cells per dish. Good proposes culturing the cells in selection media until colonies form and then isolating the colonies using cloning rings (¶¶ 167 and 169).

As opined by Dr. Robl, such a procedure would likely be unsuccessful. First, as noted by Good, the transfection procedure will produce many neomycin resistant cells,

but only a fraction of those cells will have been correctly targeted (¶ 109). As a result, the plating density employed (500,000 cells/dish) is too high to allow for efficient isolation of individual colonies, as the plated cells would be essentially confluent. In addition, fibroblasts are motile cells that migrate in culture dishes, further complicating the isolation of any individual colonies under the conditions proposed in the Good reference. Because the cells are motile and would be essentially confluent, colonies isolated using cloning rings, as taught in Good, would be mixed, i.e., the colonies would contain targeted and non-targeted cells. Southern blot genotyping on such a mixed colony could produce a positive signal if some correctly targeted cells were present. There would, however, be no guarantee that fetuses produced using cells from this mixed colony would be transgenic, as a large proportion of the cells would not harbor a prion knockout.

Dr. Robl further states that Good's proposed primary screening method for identifying correctly targeted prion knockout cells is incompatible with fibroblasts, which senesce (Dr. Robl's Declaration ¶ 3). As described in paragraph 169, Good proposes duplicating each colony and then performing PCR followed by Southern blotting for genotyping. Good, however, provides no teachings on how to obtain sufficient DNA from selected colonies to perform PCR followed by Southern blotting. As noted by Good, the proposed cloning process requires many population doublings leaving few doublings left for expansion (¶ 171), and in Dr. Robl's opinion, the targeted cells would senesce before a sufficient quantity of DNA was obtained to perform the proposed genotyping step. No alternative screening procedures are disclosed.

Dr. Robl further states that Good proposes expanding the colonies for freezing but fails to provide any methods for carrying out such a process, and because the cells in Good are near senescence, it would be difficult or impossible to produce adequate cells for freezing as the cells in Good's technique would not be dividing.

Dr. Robl further states that Good provides insufficient guidance for the second targeting step, which would be necessary to produce homozygous KO cows (Dr. Robl's Declaration ¶ 4). As noted by Good, a second round of targeting is typically inefficient

because the second vector often recombines with the previously targeted allele and the use of the same selection marker will not distinguish between heterozygous and homozygous cells (¶ 206). While Good does suggest use of a second marker to determine whether the second vector has been successfully incorporated into a cell, Good fails to provide any guidance on how to prevent retargeting of the first allele. In these experiments, retargeting of the first allele occurs frequently, resulting in a failure to produce homozygous knockouts or requiring an impractical number of transfections.

Thus, Good does not disclose a method that, in the absence of undue experimentation, would result in the production of a transgenic bovine cell or bovine fetus as instantly claimed, and claims 32 and 37 and their dependent claims, which require production of PrP knockout bovine cells or fetuses, are therefore distinct from the disclosure of Good. Moreover, since Good fails to produce a PrP knockout cell or bovine, or provide an enabling method to do so, Good also fails to disclose such cells or bovines as claimed in claims 1 and 25. These claims therefore are also distinguishable from the Good disclosure.

Indeed, Applicants submit that the facts of the present case are more akin to the facts of *In re LeGrice*, 301 F.2d 929 (CCPA 1962) (copy enclosed) than those of *In re Crosby*. In *LeGrice*, the applicant sought patents for two varieties of roses. Pictures of both of the claimed roses were printed in catalogs more than one year prior to the filing date of the applications. The prior publication of the exact roses later claimed was not, however, a statutory bar because the published pictures did not enable production of the claimed roses. That is, publication of the very subject matter being claimed – subject matter that was in no way distinguishable from the claimed invention -- was not anticipatory due to the lack of an enabling disclosure. In so holding, the court provided a general rule: "descriptions ... in order to bar the issuance of a patent, must be capable, when taken in conjunction with the knowledge of those skilled in the art to which they pertain, of placing the invention in the possession of those so skilled." *Id.* at 944. Good in combination with the general knowledge has not placed the presently claimed

invention in the possession of those skilled in the art. Good therefore cannot be anticipatory to the instant claims. This basis of the rejection may be withdrawn.

With respect to the second objection, Applicants direct the Examiner to the discussion above regarding the enabling nature of Applicants' specification. In view of that discussion, Applicants submit that they have described in detail how they have successfully produced PrP knockout cows in both the September 2006 Declaration of Dr. Kuroiwa and the presently submitted June 2007 Declaration of Dr. Kuroiwa.

Moreover, as stated by Dr. Robl, the processes described in the present application used to successfully produce a homozygous prion knockout bovine are distinct from those of Good (Dr. Robl's Declaration ¶ 5). In particular contrast to Good, the specification teaches dilution plating of 10,000,000 electroporated cells into sixty 24-well plates, resulting in a much lower initial density of cells (~7000 per well) compared to Good (~500,000 per well) and allowing the formation of individual colonies in a well (page 56, lines 5-26). This procedure allows for isolation of a colony of homogenous cells.

In addition, the specification provides a PCR-based screening method for targeted integrations that does not rely on Southern blotting. This screening method employs two sets of primers for heterozygous targeting (F7 and R7 and F10 and R10) and one set of primers for homozygous targeting (F14 and R14) followed by electrophoretic analysis of the PCR products (page 56, line 28 to page 57, line 25 and page 59, line 21 to page 60, line 18). These methods do not require multiple population doublings to produce sufficient DNA, unlike the Southern blotting approach of Good.

In further contrast to Good, the specification illustrates in Figs. 44A and 44B two prion protein knockout vectors used to produce homozygous knockout cells. A vector having a neomycin resistance gene was employed to form heterozygous PrP KO cells, and a vector having a puromycin resistance gene was employed to form homozygous PrP KO cells. Experimental evidence shows that the neo vector preferentially targeted one allele, and the puro vector preferentially targeted the other. This basis for the rejection may also be withdrawn.

Finally, with respect to the third objection, Applicants request that the Office reconsider its position on the procedural posture of this case. Both U.S. Application No. 10/971,541, which is a continuation of Good, and the present application are pending, and the claims in the Good continuation have not been allowed. Indeed, as noted in the previous reply, the Office has consistently rejected the claims in Good for lack of enablement (see final Office action issued on January 9, 2007). As noted in M.P.E.P. § 2303.01, in such a situation, request for an interference is inappropriate. Applicants submit that the proper procedure is to issue the present application and await the completion of examination in the Good continuation application before considering the propriety of an interference (see Example 2 of M.P.E.P. § 2303.01). This final basis for the anticipation rejection may be withdrawn.

CONCLUSION

Applicants submit that the claims are in condition for allowance, and such action is respectfully requested. Enclosed is a petition to extend the period for reply for three months, to and including June 1, 2007. If there are any charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

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